Investigation of Human Urine for Genomic Sequences of the Primate Polyomaviruses Simian Virus 40, BK Virus, and JC Virus

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Recent reports of the detection of simian virus 40 (SV40) nucleotide sequences in ependymomas, choroid plexus tumors, osteosarcomas, and mesotheliomas have raised the possibility that SV40, which naturally infects Asian macaques, is circulating among humans. This possibility was examined by performing polymerase chain reaction assays on urine samples of 166 homosexual men, 88 of them human immunodeficiency virus (HIV)–seropositive, for genomic sequences of SV40 as well as of human polyomaviruses BK virus (BKV) and JC virus (JCV). Tests with masked urine specimens spiked with SV40-transformed cells were included to monitor the SV40 assay. SV40, BKV, and JCV sequences were identified, respectively, in 0, 14%, and 34% of the urine specimens. JCV viruria was far more common (37%) than BKV viruria (5%) in HIV-seronegative persons. HIV infection and more severe immunosuppression were associated with a higher frequency of BKV viruria. In summary, SV40 viruria was not detected among homosexual men who shed human polyomaviruses at a high frequency.

DNA sequences of simian virus 40 (SV40), a natural infection of Asian macaques, were recently reported to be present in several rare human cancers, including ependymomas and choroid plexus tumors (brain tumors) [1], which typically occur in children <5 years old, osteosarcomas [2], which occur most commonly in the first and second decade of life, and in pleural mesotheliomas [3], which are asbestos-related cancers primarily affecting men aged >50. In addition, infectious SV40 was isolated from one ependymoma tissue [4]. In contrast to the above findings, our study of mesotheliomas in US residents failed to detect SV40 in tumor tissues [5].

It has remained unclear how the reported SV40-positive patients might have acquired SV40 infection [1–3]. There is no known animal reservoir of SV40 in the United States. The only documented exposure of US residents to SV40 occurred between 1955 and 1963, principally to recipients of the inactivated poliovirus (Salk) vaccines [6, 7]. During this time period, experimental and licensed viral vaccines that used virus pools prepared in macaque kidney cultures were inadvertently contaminated with SV40. Federal regulations put into effect after the discovery of SV40 make it unlikely that any product administered after 1963 would have been contaminated with SV40 [7].

The US patients recently reported to have SV40-positive ependymomas, choroid plexus tumors, and osteosarcomas were born many years after 1963 and were therefore at no risk of acquiring SV40 from contaminated vaccines. It has been suggested, as one possible explanation of these findings, that SV40 might have become a human-adapted virus, capable of circulating efficiently in communities by person-to-person transmission, without the need for a nonhuman animal reservoir [1]. In this report, we have examined this possibility by testing urine specimens from immunosuppressed and nonimmunosuppressed persons for genomic sequences of SV40 as well as of the human polyomaviruses BK virus (BKV) and JC virus (JCV). The human polyomaviruses are frequently shed in urine [8]. It seemed probable that SV40, which is closely related to BKV and JCV and which remains latent in the kidneys of its natural simian hosts, would be found in human urine, especially in urine of immunocompromised persons, if it were circulating in the communities.

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Methods

Urine specimens. Urine specimens from 88 human immunodeficiency virus (HIV)–positive and 78 HIV-negative homosexual men in Washington, DC, and New York City were tested. These urine specimens were collected between 1986 and 1988 as part of a natural history study of HIV infection in homosexual men and were stored at −70°C [9]. Data on HIV serostatus and CD4 cell counts of the urine donors, obtained at the same visit when urine was collected, were available [10]. The subjects were mostly white (91%). The age distributions of the HIV-positive and HIV-negative

men were similar (age range, 28–69 years; median age, 39 for HIV-positive and 38 for HIV-negative men).

The urine samples were processed by an independent laboratory under contract to the Viral Epidemiology Branch of the National Cancer Institute (NCI). The amount of urine available was 5–10 mL for 140 men, 3–5 mL for 23 men, and <3 mL for 3 men. The specimens were centrifuged at 49,000 *g* for 4 h to pellet cells as well as free virus. The pellet was resuspended in 500 μL of distilled water by vortexing, and the tube was placed in an ultrasonic water bath to disrupt the cells. Each specimen was then aliquoted into three tubes and stored at −70°C.

Masked SV40-positive controls. To ensure that the tests were able to detect SV40, masked SV40-positive controls were prepared at the Johns Hopkins University laboratory by spiking a normal urine sample with a known number of SV40-transformed human cells, as follows. SV40-transformed human fibroblast WI26 cell line, purchased from the American Type Culture Collection (ATCC CCL95.1; Rockville, MD), was cultured in the laboratory. The cultured cells were trypsinized and counted with a hemocytometer, and 40,000 cells were placed into each of several 10-mL tubes that contained 6 mL of normal urine. These tubes were transported to the independent laboratory under NCI contract, where they were masked and processed in the same way as the urine samples from patients. About 10% of the samples sent to the Johns Hopkins University laboratory for polymerase chain reaction (PCR) assays were masked SV40-positive specimens. As described below, 5 μL of a 1 in 2 dilution of the processed specimen was used for each PCR assay. It was estimated that for the masked SV40-positive controls, that would represent 200 SV40-transformed cells.

Negative controls. Every 12th specimen in the test (column 7 in the filters in figure 1) was a negative control, consisting of 5000 K562 human cells.

PCR amplification and identification. PCR amplifications were done as described [5] in a 96-well thermocycler (Perkin-Elmer Cetus, Norwalk, CT). Amplification products were transferred to Biotrans(+) nylon filters (ICN, Costa Mesa, CA) and identified with biotinylated probes in the Amersham enhanced chemiluminescence system.

Briefly, 100 μL of the specimen was digested with an equal amount of 400 μg/mL proteinase K; 5 μL of the digest was used for each PCR reaction. Two PCR amplifications were done: for SV40, using primers SV.For3 and SV.Rev, which amplify a 105-bp segment of the T antigen region of SV40 [1], and for BKV and JCV, using primers PEP-1 and PEP-2, which amplify a conserved, 218-bp segment of T antigen of both viruses [11]. SV40 was identified with probe sequence GGAAAGTCCTTGGGGTCTTCTACC [2], BKV with probe BEP-1, and JCV with probe JEP-2 [11].

Statistical analysis. The DNA results were summarized in contingency tables and analyzed by standard χ^2 statistics, including Fisher's exact test, or by the extended Mantel-Haenszel χ^2 method, to test for trends [12] associated with HIV infection and CD4 cell numbers. Multivariate analyses were done with logistic regression models, using the likelihood ratio test to measure the significance of individual parameters [13].

Results

The patterns of hybridization with SV40, BKV, and JCV, and the lack of cross-hybridization between the virus hybridization controls, are illustrated in figure 1.

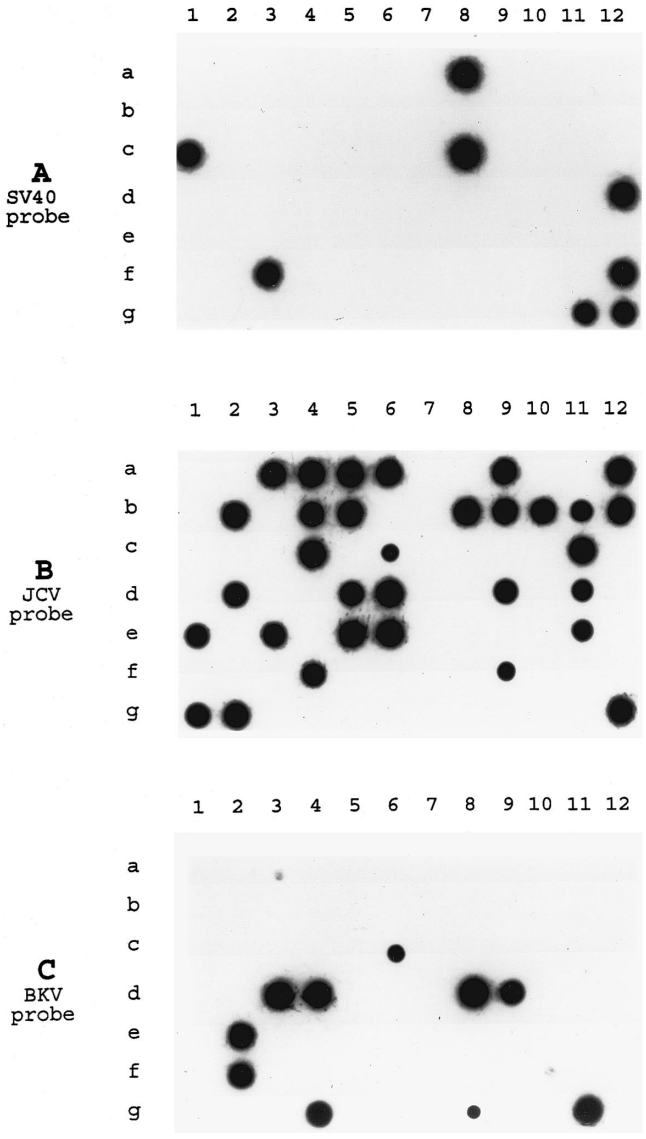

Figure 1. Hybridization of filters containing 75 specimens with biotinylated probes to SV40 (filter A), JCV (filter B), and BKV (filter C). Specimens are in same relative position on 3 filters, except for G11 and G12, which are hybridization controls. G11 and G12 on filter A contain amplicons of SV40 and on filters B and C contain amplicons of BKV (G11) and of JCV (G12). Specimens in column 7 are negative controls. 6 specimens that hybridize with SV40 in filter A are all masked SV40-positive controls. Specimens A3, C6, and D9 contain both BKV and JCV.

BKV sequences were identified in 23 (14%) and JCV sequences in 56 (34%) of the 166 urine samples. Four specimens contained both BKV and JCV (3 of these are shown in figure 1). BKV shedding was uncommon in HIV-negative donors (5%), but it increased to 22% in HIV-positive donors (P = .002; table 1). Moreover, in HIV-positive subjects, prevalence of BKV DNA increased incrementally with greater immunosuppression, from a low of 18% in subjects with >500 CD4

Table 1. SV40, BKV, and JCV sequences in human urine specimens by degree of immunosuppression.

Urine donors	No. tested	SV40, no. positive	BKV		JCV	
			No. positive (%)	OR (95% CI)	No. positive (%)	OR (95% CI)
HIV-seronegative	78	0	4 (5)	1	29 (37)	1
HIV-seropositive						
CD4 cell count >500	33	0	6 (18)	4.1 (0.9–21.1)	11 (33)	0.8 (0.3–2.2)
CD4 cell count 200–500	39	0	8 (21)	4.8 (1.2–23.0)	9 (23)	0.5 (0.2–1.3)
CD4 cell count <200	15	0	4 (27)	6.7 (1.1–40.1)	7 (47)	1.5 (0.4–5.1)
All seropositive*	88	0	19 (22)	5.1 (1.6–21.4)	27 (31)	0.7 (0.4–1.5)

NOTE. OR, odds ratio; CI, confidence interval.
* CD4 cell counts were not available for 1 HIV-seropositive donor.

cells to a high of 27% in subjects with <200 CD4 cells. The overall relation of BKV viruria with immune status (HIV infection and CD4 cell count) was highly statistically significant ($P = .004$), although this trend was not significant within the HIV-positive stratum.

The pattern of JCV shedding was quite different from that of BKV. In HIV-negative patients, JCV shedding was very common (37%) and far more frequent than BKV shedding (37% vs. 4%; $P < .001$). However, JCV shedding was not influenced by HIV infection status or by the degree of immunosuppression in HIV-positive persons. Adjustment for age in multivariate analyses did not affect these findings for JCV or for BKV.

In contrast to the results with BKV and JCV, SV40 sequences were not detected in any of the 166 urine specimens. All of the 17 masked SV40-positive controls (6 of them shown on filter A in figure 1) were positive for SV40 and negative for BKV and JCV. All of 17 negative controls were negative for all three viruses.

Discussion

The main objective of our study was to investigate the possibility that SV40 might be circulating in human communities by person-to-person transmission and independently of any animal reservoir. We examined urine specimens from immunosuppressed and nonimmunosuppressed patients for genomic sequences of SV40 as well as of human polyomaviruses BKV and JCV. The PCR assay for SV40 was shown to detect SV40 consistently in masked normal urine samples spiked with SV40-transformed cells. The results of the urine examinations were unambiguous. While 75 (45%) of the 166 urine samples tested contained sequences of either BKV or JCV or both viruses, none were positive for SV40. Thus, we were unable to find evidence in support of SV40 circulating in human communities. In a previous study, we were not able to detect SV40 sequences in mesothelioma tissues or to identify antibodies to SV40 in a significant proportion of mesothelioma or osteosarcoma patients [5].

Both BKV and JCV were readily detected in the urine specimens. However, the two viruses showed different patterns of viruria. BKV shedding was infrequent in HIV-seronegative but common in HIV-seropositive patients, and in the latter group, the proportion of patients shedding BKV increased incrementally with greater immunosuppression. The same relationship between immunologic status and BKV viruria was reported by Markowitz et al. [14]. In contrast, JCV shedding was detected in about one-third of the patients regardless of HIV infection status and degree of immunosuppression, confirming a previous observation that in immune competent persons, JCV is shed more frequently than BKV [15].

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Plasma Levels of Monocyte Chemoattractant Protein-1 but Not Those of Macrophage Inhibitory Protein-1α and RANTES Correlate with Virus Load in Human Immunodeficiency Virus Infection

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Plasma levels of proinflammatory cytokines, cytokine inhibitors, and the β chemokines RANTES, macrophage inhibitory protein (MIP)-1α, and monocyte chemoattractant protein (MCP)-1 were studied in relationship with virus load in 40 patients exhibiting plasma levels of HIV RNA ranging between undetectable and levels $>10^6$ copies/mL. Mean plasma levels of MCP-1 were increased in patients with high virus load compared with HIV-seropositive subjects with undetectable plasma viral RNA and healthy controls. MCP-1 levels were directly correlated with plasma levels of HIV RNA. No correlation was observed between virus load and plasma concentrations of MIP-1α and RANTES. The results suggest that low rates of viral replication in vivo are not dependent on increased production of the suppressive chemokines RANTES and MIP-1α. Since MCP-1 up-regulates viral replication in vitro, the results may suggest a role for MCP-1 in triggering viral replication in HIV disease.

Persistent immune activation is a common immunopathogenic feature of human immunodeficiency virus (HIV) infection. Circulating levels of the proinflammatory cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α), of the IL-1 antagonist IL-1ra and the soluble forms of TNF receptors, and of soluble CD14 (sCD14) are increased in HIV-infected persons [1–3]. The monocytic cytokines IL-1 and TNF-α contribute to the pathogenesis of AIDS by inducing virus expression in infected cells [4]. Elevated serum levels of the soluble forms of type I and II TNF-α receptors (sTNFR-I and sTNFR-II) have been correlated with rapid disease progression [5], and serum concentrations of sCD14 were found to be related to the clinical stage of the disease [3].

The β chemokines RANTES, macrophage inhibitory protein (MIP)-1α, and MIP-1ß have recently been identified as soluble suppressors of HIV infection by macrophage-tropic strains in vitro [6]. In contrast, monocyte chemoattractant protein (MCP)-1 was shown to up-regulate HIV replication in cultures of CD8-depleted peripheral blood mononuclear cells of HIV-infected patients [7]. Few data are available on plasma levels of chemokines in HIV infection. In two studies, the serum levels of RANTES, MIP-1α, and MIP-1β did not differ between patients with AIDS and patients with nonprogressing HIV infection [8, 9]. A recent report indicated that, following initiation of antiprotease-based antiretroviral therapy in patients with advanced disease, the decrease in plasma HIV RNA levels was associated with increased concentrations of MIP-1α, MIP-1β, RANTES, and IL-16 and a significant decrease in plasma levels of MCP-1 [10].

In the present study, we investigated plasma levels of proinflammatory cytokines TNF-α and IL-6, of circulating cytokine

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